



## Investigation of cytokines and herpes simplex virus in recurrent abortion in pregnant women

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### Abstract

This study aimed to investigate the existence of Herpes simplex virus (HSV) type 1 and type 2 in abortion women as well as to detect the role of cytokines in the induction of abortion. Over the period from November (2019) to February (2020), a total of 68 cases of abortion women Collected from AL-Zahraa Teaching Hospital in AL-Najaf city, and the women's generating of children teaching hospital Kerbala. Furthermore, the control group relied on 20 healthy pregnant women without any evidence of chronic inflammatory disease, two types of cases with ages ranging from 16-40 years. Blood samples were obtained from abortion women and healthy controls were tested for HSV-1 and HSV-2 tested first on Rapid ICT Kits. Of the 68 samples, 9(13%) were positive, and 59(87%) were negative in Rapid *Immunochromatographic Test (ICT)*, While the results of pregnant women's investigation showed Nil(0%) negative result for every 20 samples these findings were then verified by using Enzyme-Linked Immunosorbent Assay (ELISA) technique to investigate HSV type 1 and type 2 where attained 38(56%) positive samples in the aborted women and 8(40%) positive samples in the pregnant women respectively. Collected samples from abortion women and control estimate immunological level (IL-2, IL-10 & TFN- $\alpha$ , C3) Used the ELISA and radial immunodiffusion method. The results revealed the sensitivity of rapid (ICT) compare to the ELISA technique was 11 %. This ratio indicates the true positive HSV infection and the specificity was 92% indicate to the true negative infection with HSV. And the positive cases of HSV-1 and HSV-2 in aborted women were recorded 27(40%) at the first trimester. In another side, the other cases of abortion occurred in the second and third trimester were reached (16/68) and (1/68) respectively. The Cytokine tests. High significance ( $p < 0.05$ ) of IL-2 in aborted + HSV women ( $3.994 \pm 0.9354$ ) and aborted -HSV women ( $4.655 \pm 1.423$ ) compared to control pregnant women ( $2.903 \pm 0.7484$ ) whereas there was a non-significant difference between aborted + HSV women and aborted -HSV women. The results of this study revealed a high level of IL-2 in the serum of aborted women as positive and negative for HSV compared with pregnant women. While the result of IL-10 level showed great significance ( $p < 0.05$ ) in aborted +HSV women ( $2.709 \pm 0.3877$ ), aborted -HSV women ( $2.905 \pm 0.3368$ ) compared with control (pregnant) women ( $2.267 \pm 0.2332$ ) But there is no -significant difference ( $p < 0.05$ ) among aborted +HSV women and aborted -HSV . On the other hand Results showed great significance ( $p < 0.05$ ) of TNF- $\alpha$  level in aborted +HSV women ( $4.859 \pm 0.6083$ ), aborted -HSV women ( $4.770 \pm 0.4548$ ) compared with control (pregnant) women ( $4.841 \pm 0.4153$ ) while there was non-significant difference ( $p < 0.05$ ) among aborted +HSV women and aborted -HSV women. In the present study, the results detected high significance ( $p < 0.05$ ) in the levels of C3 for aged group  $\geq 42$  ( $85.80 \pm 24.63$  mg/dl) in comparison to the control ( $79.10 \pm 4.890$  mg/dl).

**Keywords:** Abortion women, Herpes Simplex Virus type 1 and type 2, cytokines, ELISA, Rapid (ICT)

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### INTRODUCTION

Abortion meaning, end case of pregnancy after eradicating a fetus or embryo before. It can be live outside from the uterus (Grimse and Stuart, 2010). Maternal morbidity and mortality linked to complications of unsafe abortion have been recognized as major general health problems. Moreover, all unsafe abortions occur in developing countries, where the ninety - eight

percentage of abortion-related to death. About fifty-six million abortions are turned up each year in the world with a less under half achieved unsafely (WHO, 2012, Yezlankyzy & Niazbekov, 2015).

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Recurrent pregnancy loss (RPL) causes maternal infections transportable in utero at the different cases of gestation can be caused by a wide range of organisms, represented by herpes viruses, cytomegalovirus, rubella virus, and *Toxoplasma gondii* (Hussan, 2013; Ghasemina, et al. 2016). It can be known the causes of RPL related to the abnormalities of chromosomes and metabolites, uterine anomalies and immunological factors (Cao et al. 2014). According to ASRM, recently has redefined RPL as the occurring two or more lost of pregnancy (Naematzadeh *et al.*, 2016; Kamali et al. 2018). Herpes simplex virus (HSV) is considered a frequent broad spread disease in people, that is effect about 60%-95% of the mature population in the world (Marchi et al. 2017), another side HSV are DNA viruses belonging to Alphaherpesvirinae subfamily of the Herpesviridae family, they are represented the agents responsible for a wide range of infections in people, include genital and oral mucocutaneous damages but in some rare life-threatening cases such as fulminate encephalitis (Arshad et al. 2019), the models of recent to evaluate the infection by the herpes virus. Globally, the prevalence of herpes one type (HSV-1) in humans aged 0-49 years was 3.7 billion (or 67% of the world population) while the prevalence of two types (HSV-2) in humans aged 15-49 years was up to 400 million (or 11.3% of the world population) (Thellman and Triezenberg, 2017). Cytokines are immunomodulatory proteins which particularly significant for to the immune response, innate and adaptive that depending on their chemical inflammatory reaction, broadly can be divided into pro-inflammatory and anti-inflammatory cytokines formed by T-helper cell type 1(Th1) and T-helper cell type 2(Th2) (Kaur and Kaur, 2011). Immune response to cytokinesis among the particles plays a vital role in pregnancy also are expressed maternal-fetal interaction by immune cells such as trophoblast, on the other hand, cytokines are participated in recognized of origin T-helper cell (Whitcomb et al. 2008). The existence of antibodies are produced to both HSV-1 and HSV-2 at the first pregnancy, So in first genital HSV disease in late pregnancy represent 30%-50% risk of an infant's infection but in early pregnancy a risk less than 1% (Xu et al. 2006).

## MATERIALS AND METHODS

### Patient Groups

All samples collected from Al-Zahraa Teaching Hospital in AL-Najaf city, and the women's generating of children teaching hospital Kerbala, during the period from November (2019) to February (2020). Total of 68 women, they have a recurrent abortion, also other 20 cases normal pregnancies represent control with an aged range between (16-42) years. All samples partitioned to four groups according to the age. considered as pregnant women and have been

partitioned into two groups, the first group includes twenty samples and represents control and the other groups comprise sixty-eight samples and represent women who suffer from recurrent abortion.

### Blood Sample Collection

The collection of blood samples were done in the blood bank of AL-Zahraa hospital in AL-Najaf city, about five millilitres of fresh blood was drawn from each patient via vein puncture then collected in the two groups of tubes, the one contains anti-coagulant to evaluate complete blood count and the other group Without the anti-coagulant to be used as a simple tube for sera preparation, they were centrifuged for five minutes at three thousand round per minute to separate serum, then transport into other tubes where the serum samples transfer into sterile test tubes utilizing micropipette with sterile disposable tips. Every sample was labelled and given a serial number along with the name of the patient, and the serum samples were then frozen at (-20 ° C) until used.

### Detection of HSV IgM and IgG by RapidTest Cassette (Cit EST, CANADA)

HSV IgM/IgG rapid test cassette (whole blood/serum/plasma) is a qualitative, lateral flow immunoassay for the detection of IgG and IgM antibodies to HSV-1 and HSV-2 in whole blood, serum or plasma specimens. In this test, mouse anti-human IgG and goat anti-human IgM are covered in test area test line regions. During testing, the whole blood, serum or plasma specimens react with HV-1 and HSV-2 antigens Particulate coated in the test strip. Then, the mixture migrates on the membranes by capillary action and respectively. The presence of a cored line in the test line region indicates a positive result for HSV-1 and HSV-2 infection, Whereas, its absence indicates a negative outcome for the infection. To serve as a procedural control, a coloured line will always appear in the respective control line reigns of all the two strips indicating that adequate specimen volume has been added and membrane wicking has taken place.

### Testing Procedure

Enables the test, specimen, buffer and /or controls To reach the temperature of the room (15-30 C) before testing.

1- The test cassette and test tips removed from the sealed pouch and use it within one hour. The best results obtained if the assay is performed as soon as possible.

2- Placed the test cassette on a clean and level surface. Hold the dropper vertically, drew the specimen about 1 cm above the upper end of the nozzle as shown in the illustration below. Transferred 1 full drop approximately 20 microliters of the specimen to sample well, then added 2 drops of buffer approximately 80 microliters to sample well the time was calculated.

3- Waited 15 minutes for the coloured line to appeared and test tips.

### Detection of HSV IgM Antibodies in Human Serum by ELISA kit. (Vircell, Spain)

The ELISA approach is based on the reaction of the antibodies in the sample examined with the antigen adsorbed on the polystyrene surface. washed unbound immunoglobulins. In a second step, an enzyme-labelled anti-human globulin binds the complex antigen-antibody. The bound conjugate is developed after a new washing step with the help of substratum solution (TMB) to make a blue-coloured soluble product turning into yellow after adding the acid stop solution.

#### Procedure

The test was planned as per the Instructions to the manufacturer. Test of These was calculated by equation Defined in Kit.

## RESULTS

Calculate the mean of O.D for cut off the serum

Antibody index = (Sample O.D / Cut Off serum mean O.D) × 10.

#### Detection of Cytokines

##### Estimation of IL-2 by ELISA Kit (Elabscience, USA)

The sandwich-ELISA principle is used in this ELISA kit. The micro ELISA plate contained in this kit is pre-coated with a human IL-2 specific antibody, the standard Or samples are applied to micro ELISA plate wells and combined with the appropriate antibody. Biotinylated detection antibody specific to human conjugate IL-2 and Avidin-Horseradish peroxidase (HRP) are Then add to every well, respectively and incubated. Only those wells containing human IL-2, biotinylated antibody detection, and conjugate Avidin-HRP, should appear blue. The reaction of the enzyme-substrate is terminated by adding the stop solution and the colour turns yellow. The spectrophotometrically is measured with optical density (OD). At 450 nm wavelength  $\pm 2$  nm The OD-value is proportional to human IL-2 concentration. The concentration of human IL-2 in the samples can be calculated by comparing the sample OD with the standard curve.

#### Procedure

The test was planned as per the Instructions to the manufacturer. Test of These was calculated by equation Defined in Kit.

For each standard and sample, average the duplicate readings, then remove the normal optical density average zero. Plot a logistic curve on a log-log graph board with four parameters, with a normal concentration on the x-axis and y-axis OD values. If the samples have been diluted, then He must multiply the dilution factor calculated from the standard curve. If the sample's OD reaches the standard curve's upper limit,

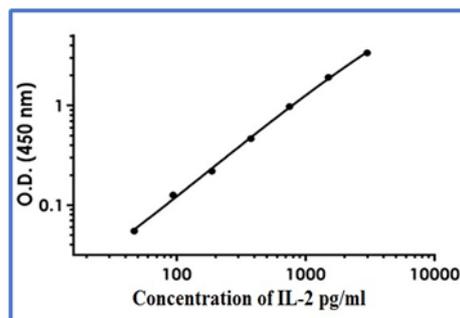


Fig. 1. Standard Curve of IL-2 (Elabscience, USA)

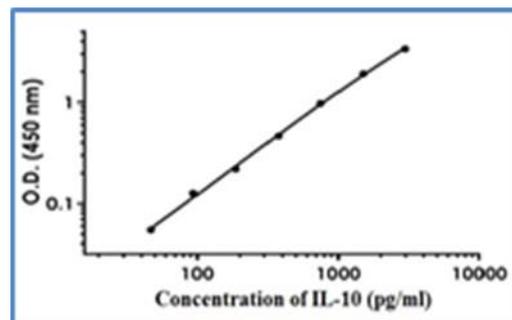


Fig. 2. Standard Curve of IL-10 (Elabscience, USA)

then you can re-test it with sufficient dilution. The true concentration is the measured concentration which is multiplied by the dilution factor. Fig. 1.

##### Estimation of IL-10 by ELISA Kit (Elabscience, USA)

The working principle of this ELISA group is the ELISA sandwich, which is the same Principle OF IL-2 ELISA Kit but the difference only in the coating of the small ELISA plate presented in this group previously with an antibody specific to the human IL-10.

#### Procedure

The test was planned as per the Instructions to the manufacturer. Test of These was calculated by equation Defined in Kit.

#### Results Calculation

For each standard and sample, average the duplicate readings, then substrate the average normal optical density zero. Plot a logistic curve on a log-log graph board with four parameters, with a normal concentration on the x-axis and y-axis OD values. If the samples have been diluted, then The concentration measured from the standard curve will multiply by the dilution factor. If the sample's OD reaches the standard curve's upper limit, then you can re-test it with sufficient dilution. The real concentration is the concentration measured which is multiplied by the dilution factor. Fig. 2.

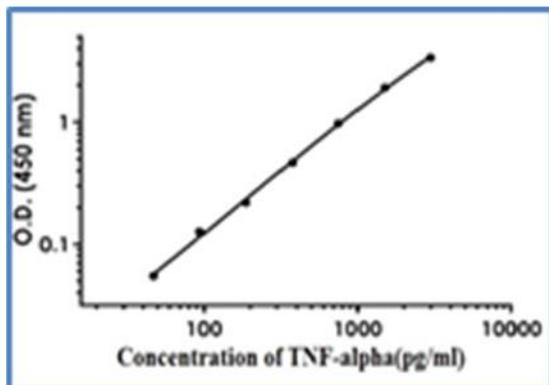


Fig. 3. Standard curve of TNF- α (Elabscience, USA)

**Estimation of Tumor Necrosis Factor - Alpha (TNF-α) by ELISA Kit (Elabscience, USA)**

The principle of this Interleukin was similar to the complement IL-10 principle excepted the antibodies specific to human TNF-α.

**Procedure**

The test was planned as per the Instructions to the manufacturer. Test of These was calculated by equation Defined in Kit.

For each standard and sample, average the duplicate readings, then substrate the average normal optical density zero. Plot a logistic curve on a log-log graph board with four parameters, with a normal concentration on the x-axis and y-axis OD values. If the samples have been diluted, then The concentration measured from the standard curve will multiply by the dilution factor. If the sample's OD reaches the standard curve's upper limit, then you can re-test it with sufficient dilution. The real concentration is the concentration measured which is multiplied by the dilution factor.

**Complement (C3)**

The determination of the (C3) protein by (RID) radial immunodiffusion plate was performed according to the manufacturing company (LTA s. r. l, Italy).

**Procedure According to enterprise manufacturer**

- 1- The plate was removed from its container and left for a few minutes at room temperature for any excess water in the well to evaporate.
- 2- Five microlitter of sample and controls has filled the wells and waited to completely adsorbed before handed the plate.
- 3- The plate was put in a moist chamber and locked. Then incubated for 72 hours.

**Results Calculation**

The concentration value corresponding to the diameter of the precipitated ring was read on an enclosed reference table, for each complement (C3).

**Table 1.** Demonstration of Sensitivity, Specificity and prevalence of Disease

	Disease Number	None Disease Number	Total Number
No. Positive	A true positive	B, false positive	T-test positive
No. Negative	C, false negative	D, true negative	T-test negative
	T Disease	T Non-Disease	Total

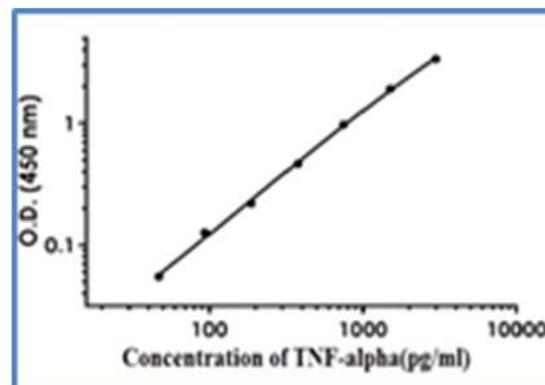


Fig. 4. Distribution of samples among aborted and control pregnant women

**Sensitivity and Specificity**

Estimated the sensitivity and specificity depending on the golden standard which was ELIZA Test according to (Al-Khilkhali,2019) as shown in **Table 1**.

Sensitivity=  $a/a+c$  ×100

Specificity =  $d/b+d$  ×100

a: true positive, d: true negative, b: false positive, c: false negative

Prevalence of disease:  $T \text{ disease} / \text{Total} \times 100$

**Statistical analysis**

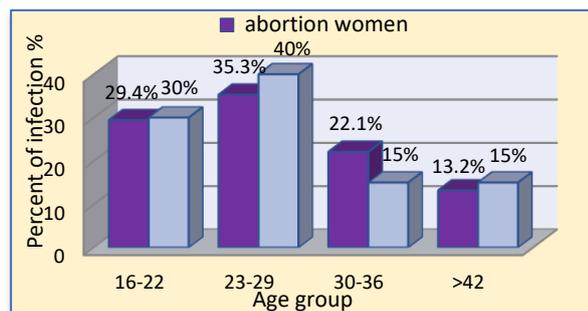
In the current study, the data were undergoing analyzed by using the statistical program Graph pad prism (GPP) version 5, also use of Microsoft Excel program and compared the results at the lowest limit of significance Taking P-value below 0.05 ( $p<0.05$ ). (Obaid and Juma, 2017).

**Samples Collection**

All specimens, were collected that reach 88 samples from AL Zahra Teaching Hospital in AL-Najaf city, and also the teaching hospital of women and generating of children in Karbala. and its partitioning into two parts, the one part represented cases of abortion women by 68 (77%) samples while the other part represented by 20(23%) pregnant samples that can be considered as a control group to compare and to demonstrate that can be shown in **Fig. 4**.

**Distribution according to the age group of aborted and pregnant women.**

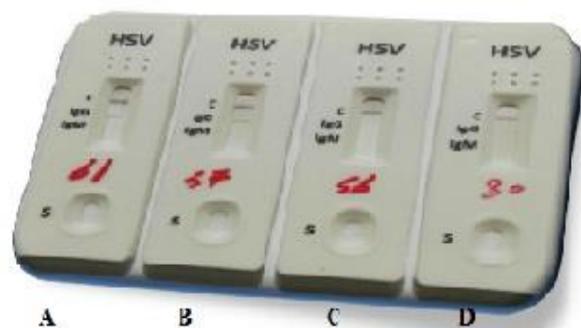
The current study showed that the Samples were distributed according to age as follows: 20 (29.4%) at 16-22 years, 24 (35.3%) at 23-29 years, 15 (22.1%) at 30-36 years and 9 (13.2%) at ≥ 42 years. On the other hand, the pregnant women can be demonstrated as 2 (30%) at 16-22 years, 8 (40%) at 23-29 years, 3 (15%) at 30-36



**Fig. 5.** Distribution, by age group, of aborted and pregnant women

**Table 2.** Distribution of abortions and control (pregnant) women by age group

Age group	Aborted women No	Percent %	Pregnant women No	Percent %
16-22	20	29.4	6	30
29-23	24	35.3	8	40
30-36	15	22.1	3	15
≥42	9	13.2	3	15
Total	68		20	



**Fig. 6.** Rapid Test, demonstrate IgG, IgM, and control line regions for serum sample of HSV infection: A: abortion positive B: abortion positive C: abortion negative D: control

years and 3 (15%) at ≥ 42 years as shown in **Table 2**, **Fig. 5**. These results revealed in the aborted and pregnant women at aged 23-29 years (35.3%) and 30-36 years (22.1%) year, agreed with those (Aljumaili et al. 2013) and (A Naqid et al. 2020), they observed the highest occurrence at the same period of age. While the results in the current study disagree with the previous study advanced by (Mohammad and Salman, 2014), who cleared the most abortion at aged ≥ 42 years, the reasons that to explain the higher occurrence at age among 23 and 29 years. One reason, represented by the relation of the highest danger of transmission to the women that infected by HSV through the gestation principally at the last trimester.

The second reason, the prevalence occur from person to person by contact especially the partner without that the persons to know their state of infection, as well as the women, have the type of reproductive state at this period age .finally, an infection may have

**Table 3.** The Levels of HSV IU/ml among aborted and control (pregnant) women

Age group	No	HSV positive	Mean of a positive sample	Mean of positive control	Mean of negative control
16-22	20	9	0.801	0.719	0.275
23-29	24	16	0.770	0.888	0.377
30-36	15	9	0.891	0.537	0.471
≥42	9	4	0.757	0.511	0.406
Total	68				

**Table 4.** Rapid (ICT) and ELISA Test for diagnosis Anti-HSV antibodies in all samples

Age groups	Total No.		Rapid Test (+)		ELISA Test (+)	
	Aborted women	Control group	Aborted women	Control group	Aborted women	Control group
16-22	20	6	4	-	9	2
23-29	24	8	3	-	16	4
30-36	15	3	1	-	9	2
≥42	9	3	1	-	4	0
Total	68	20	9	-	38(56%)	8(40%)

happened via the shedding of the virus or episodes of mild outbreaks (Kriebs,2008, Wood,2011).

**Detection of Herpes Simplex Virus by Rapid Immunochromatography Test (ICT)**

For detection, anti-HSV antibodies tested first on Rapid ICT Kits. Of the 68 samples, 9(13%) were positive, and 59(87%) were negative in Rapid (ICT), While the results of the examination of pregnant women showed Nil(0%)negative result for every 20 samples these results differ from that these in ELISA assay where attained 38(56%) Positive results in aborted women and positive results of 8(40 %) in the pregnant women respectively. The differences in the results among rapid test and ELISA assay belong to the sensitivity and specificity for each one of them as shown in **Fig. 6** and **Table 4**.

**Detection of the level of HSV IU/ml among aborted and control (pregnant) women by ELISA Technique**

88 HSV antibody samples were confirmed by ELISA samples. This included the groups of abortion and pregnant women, ELISA used as a second confirmation technique, from 68 samples were screened for HSV the results showed 38 (56%) were positive for ELISA and 30 (44%) were deemed negative. The results also showed high significance (p < 0.05) in the HSV in all groups of patients in comparison to negative control while the group with aged 30-36 years appeared the highest mean level 0.891 IU/ml in comparison to other patients shown in **Table 3**. The control group examination results 8 (40%) were positive for ELISA and 12 (60%) were negative as in **Table 4**.

The current study has been appeared cases of abortion in women belong to the causative agent of HSV where reached 38 (56%) from all cases diagnosed with abortion compared with the 8 (40%) cases of pregnant women as seen in **Table 4**, these results are the rate of HSV infection is higher than that recorded 24.4% in

**Table 5.** Sensitivity and Specificity for Rapid (ICT) and ELISA Technique

	Disease No	None Disease No	Total No
Number Positive	5	3	8
	A true positive	B false positive	
Number Negative	41	35	76
	C false negative	D true negative	
	Sensitivity= a/a+c) ×100 = 11%	Specificity= d/b+d) ×100 = 92%	

Norway (Mawak et al. 2012) and higher than 34.18% which recorded by (Obaid and Juma, 2017); 16% (Sadik et al. 2012); 3.1% (Karad and Kharat, 2015); 25% (Abu-Madi et al. 2010). While lower than which recorded 100% in the Lokoja, north-central Nigeria (Kolawole et al. 2016), Benin republic; 96.6% in Benin, Nigeria, and 93.9% in Kisumu, Kenya (Iche, 2014). And also it has been evaluated that sixty-seven per cent of the population is infected with HSV-1 and eleven per cent of the population in the world are infected with HSV-2 and around thirty-three per cent of the population in the world is evaluated to own a dormant or latent infection by HSV-1, therefore the primary infection by HSV-2 or HSV-1 that occurs before the twentieth week of pregnancy may be associated with abortion. So, HSV-2 and little ratio of HSV-1 caused repeated infections in the genital tract that can be lead to abortion. two types of herpes simplex virus as type 1 (HSV-1) and type 2 (HSV-2), the first, responsible for the infection on the oral and occur commonly but, the second principally cause infection the most common in the genitals (Crimi et al. 2019), both types are more prevalent and causing human diseases broadly. Therefore, genital infection of HSV considered almost all widespread for both types of HSV between women that own a high ability to obtain infection more than of their counterparts male (Looker et al. 2015). Use of such ELISA kit in the scientific investigation has many advantages, such as speed; the simplicity of the procedure, a high degree of precision, ability to testing both antibodies and antigens; high sensitivity and cost-effectiveness (Wald and Ashley-Morrow, 2002).

#### Sensitivity and Specificity Between Rapid (ICT) and ELISA Techniques

The result from **Table 5** showed that the sensitivity of rapid (ICT) compare to the ELISA technique was 11 %. This ratio indicates the true positive HSV infection and the specificity was 92% indicate to the true negative infection with HSV. in the present work showing the high specificities and low sensitivity for rapid (ICT) represent good result for depending on an ELISA test for screening of HSV antibodies and it is considered a golden test in detection and identification of the infection with a true positive HSV infection and it is not only based on rapid analysis But it can be used as a preliminary scan for diagnosis and then confirmation with other tests such as ELISA test should also be verified.

The presence in serum and plasma of the anti-HSV antibody indicates exposure to the virus and may

**Table 6.** Distribution of Infection HSV in aborted and control (pregnant) women by trimester of pregnancy

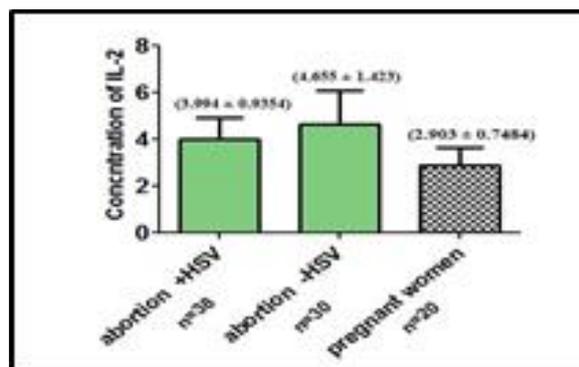
Trimester	No Aborted women	Aborted women +HSV	No Pregnant women	Pregnant women +HSV
First trimester	51	27(40%)	3	1(5%)
Second trimester	16	10(15%)	7	2(10%)
Third trimester	1	1(1%)	10	5(25%)
Total	68(77%)	38 (56%)	20(23%)	8(40%)

indicate an acute or chronic infection This is confirmed by (Loeffelholz, 2002). Rapid methods are an essential part of the diagnostic test menu to virology, significantly improving the influence of diagnostic virology on patient care. Rapid sensitivity and specificity (faster than typical cell culture).

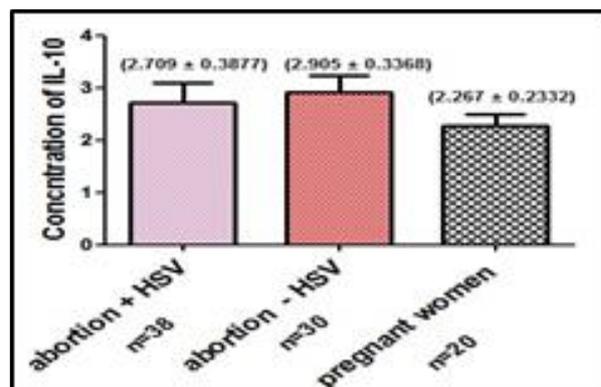
#### Distribution of HSV infection between aborted and control Women (pregnant) as according to the pregnancy trimesters

The result of aborted and pregnant women according to the stages of pregnancy trimesters occurred most of the abortions in the first trimester were reached (51/68) and the positive cases of HSV-1 and HSV-2 in aborted women were recorded 27(40%) at 1st trimester. In another side, the other cases of abortion occurred in the second and third trimester were reached (16/68) and (1/68) respectively as in Table and positive cases of HSV-1 and HSV-2 recorded 10 (15%) and 1(1%) consecutively as shown in **Table 6**, this result agreed with (Kapranos and Kotronias, 2009). They confirmed that the herpes simplex virus plays a very important role in pregnancy loss in the first trimester of pregnancy and its discovery will allow immediate antiviral treatment for a successful pregnancy in the future. As they revealed the herpes simplex virus In 41 out of 95 (43.2%) of early pregnancies and pregnancy loss in the first trimester, a very common problem occurs in more than 50% of all pregnancies, So, these results agreed with (Rasti et al. 2016) who cleared the history of the abortion among the cases group occurred in the first trimester, but the results in the current study differ from the study with (Salman, 2007) that he referred higher rate of infection by HSV in the aborted women in Second and third trimesters comparison with the first trimester. The occurrence of infection in the first trimester may belong to several causing as related in the genetically, anatomical of the reproductive also abnormalities in which the infection of virus that attracted more attention to this viral infection (Song et al. 2017).

On the other hand, the result of women who are pregnant observed High positive First Trimester samples and these results agreed with (Money and Steben, 2009) where they showed that the occurrence of infection was more in the first trimester than other both trimesters, but at the same time, the results in the current study differ from (Mohammad and Salman, 2014). certainly, increasing range among nine percent and twelve percent in adult female aged  $\leq 35$  years, but rises



**Fig. 7.** The comparison level of IL-2 in the serum of aborted +HSV women, aborted- HSV women with control (pregnant) women  
**P<0.05\*\*:** This mean high significant ( $p<0.05$ )



**Fig. 8.** The comparison level of IL-10 in the serum (pregnant) women  
**P<0.05\*\*:** This mean high significant ( $p<0.05$ )

to fifteen percent in adult female aged >40. Miscarriage will be more sorted by fetal loss or fetal abortion if it takes place after ten weeks of gestation and lost of an embryo or first-time abortion which it happens before ten weeks of pregnancy, due to elements related with the difference of each it's (Silver et al. 2011, Kolte et al. 2015).

### Immunological study

#### Measurement level Interleukin 2 (IL-2)

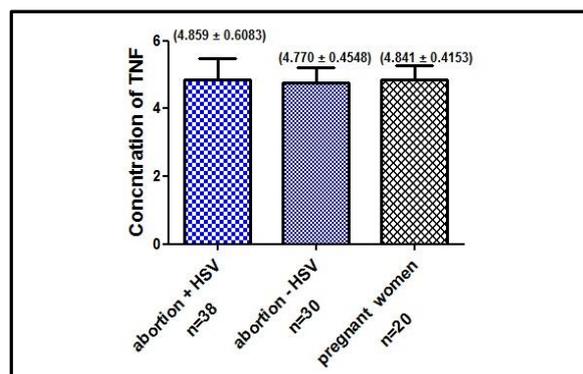
The results demonstrated high significance ( $p<0.05$ ) of IL-2 level in aborted +HSV women ( $3.994 \pm 0.9354$ ) and aborted - HSV women ( $4.655 \pm 1.423$ ) compared with control pregnant women ( $2.903 \pm 0.7484$ ) while there were non-significant difference  $p<0.05$  among aborted + HSV women and aborted - HSV women as shown in **Fig. 7**. The revealed results in this study a high level of IL-2 in the serum of aborted women as both positive and negative for HSV compared with pregnant women as shown in **Fig. 7**. These results agree with (Rezaei and Dabbagh, 2002; Pandey et al. 2005, Mohaimen and Majid, 2011) while disagreeing with (Saleh and Kharibet, 2015) where they illustrated that the low levels of IL-2 in the serum of the aborted women which suffer from recurrent abortion may be related with a deficiency of inducing effects for growth of T-cell and B-cell and that to lead to the lacking induction in the gestation to protected women immune response and may be possible the cause low levels to the IL-2 in the serum could be decreased by immunization of maternal in opposite to antigens of parental, IL-2 utilizes its influence on the response of immunity via increasing differentiation of primitive CD4+T cell for the T helper cells. IL-2 utilizes the importance of induction effect on the prostaglandin E2 (PEG2) that liberate from the tissue of chorion during the pathways of cyclooxygenase in the women. PEG2 is involved in preventing luteolysis and that is important in the helpful to the maintenance of pregnancy (Liao et al.2013).

#### Measurement level Interleukin 10 (IL-10)

The results showed great significance ( $p<0.05$ ) of IL-10 level in aborted +HSV women ( $2.709 \pm 0.3877$ ), aborted -HSV women ( $2.905 \pm 0.3368$ ) compared with control (pregnant) women ( $2.267 \pm 0.2332$ ) Whilst the difference was not significant ( $p<0.05$ ) among aborted +HSV women and aborted -HSV women as shown in **Fig. 8**. IL-10 has considered anti-inflammatory molecule serves as an agent necessary for protection and to participate in the organize of immune tolerance to the maternal and keep of natural gestation during the pregnancy (Cheng and Sharma, 2014). The results as demonstrated in **Fig. 8** in the presented study represents the high level of IL-10 in the serum of aborted women as both positive and negative for HSV compared with pregnant women that can be agreed with (Habib,2009; Mohaimen and Majid, 2011) while disagreed with (Raghupathy et al.2000, Makhseed et al. 2000, Rezaei and Dabbagh, 2002). So, the low levels of IL-10 may belong to flaw or defect in the Th1 and Th2 cells at the interface of maternal and fetal or can be caused by the collection unsuccessful to the Th2 cells at the location of implantation in the women that suffer from recurrent abortion (Michimata et al. 2003). On the other hand, the high level of IL-10 in the women with abortion can be caused by increasing gradually in the estrogens and progesterone where they become levels of these high during the period of gestation thus, these high levels caused inhibit of Th1 and induction of immunological responses which mediated by Th2 and for the same reason. (Elenkov et al.2001, Miyazaki et al.2003).

#### Measurement level Tumor necrosis factor-alpha (TNF- $\alpha$ )

Results showed great significance ( $p<0.05$ ) of TNF- $\alpha$  level in aborted +HSV women ( $4.859 \pm 0.6083$ ), aborted -HSV women ( $4.770 \pm 0.4548$ ) compared with control (pregnant) women ( $4.841 \pm 0.4153$ ) Whilst the difference was not significant ( $p<0.05$ ) among aborted +HSV women and aborted - HSV women as shown in **Fig. 9**. TNF- $\alpha$ , an inflammatory



**Fig. 9.** The comparison level of TNF- α in the serum of aborted +HSV women, aborted -HSV women with control(pregnant) women **P<0.05\*\***: This mean high significance (p<0.05)

cytokine consider one of the subpopulations that belong to the lymphocytes Th1, also it is encoded on the chromosome six, it acts as to organize some functions of the cells such as apoptosis, differentiation and proliferation of the cell, cells of macrophages consider essential producers of TNF-α as well as response to it highly (Parameswaran and Patial, 2010). In the current study, **Fig. 9** showed a slightly high level of TNF-α in the serum of aborted women as both positive and negative for HSV compared with pregnant women that can be agreed with (Rezaei and Dabbagh, 2002, Homes et al. 2003).

On the other hand, the results disagreed with (Azizieh and Raghupathy, 2015) where the study observed lower levels of TNF-α along with the period of the beginning gestation especially in both first and second trimester compared with the third trimester of pregnancy and also maybe probability that TNF-α participates in the parturition across increasing the levels of prostaglandin through altering the metabolism of its or production (Alzamil et al. 2014). The levels of TNF-α can be reversed in women that suffer from abortion and can be shown the role of this cytokine in the loss of a pregnancy as may account may be emphasis the production of the prostaglandins that cause contractions in the uterus or maybe another cause belonged the role of cytokine in the apoptosis.

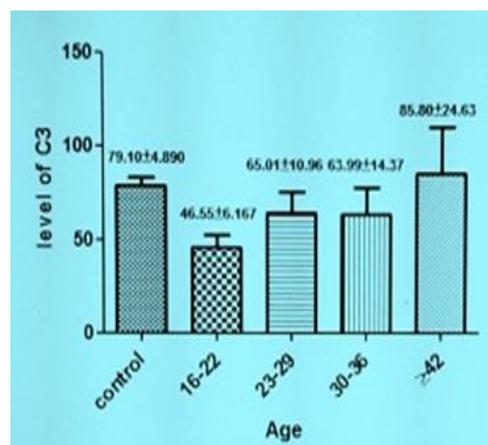
The rising of levels causes influenced on the implantation of fetal also reduces the move of blood to the fetal where causing clotting of the blood in the vessels. So, TNF-α plays an essential role in the cytokine cascades and considered a targeted drug for several of the inflammatory diseases and in the other side, the success of the gestation depends on the balance among Th1 and Th2 of cytokines that participates in the development and growth of the embryo in addition to a high concentration of TNF-α related to the complication development as gestational diabetes Mellitus (GDM) and preeclampsia (Saini et al. 2011).

**Table 7.** The Level of C3 mg/dl among aborted women in comparison to healthy control

Age	Control M±SE	16-22 M±SE	23-29 M±SE	30-36 M±SE	≥ 42 M±SE	LSD
C3						
C3mg/dl	79.10 ±4.890	46.55 ±6.167	65.01 ±10.96	63.99 ±14.37	85.80 ±24.63	0.042



**Fig. 10.** Level of C3 mg/dl in all the aborted women protein with agarose gel and visible ring around well contain the serum samples



**Fig. 11.** Level of C3 mg/dl in all the aborted women protein with agarose gel and visible ring around well contain the serum samples

**Complement C3 protein by radial immunodiffusion (RID)**

The results showed high significance (p<0.05) in the levels of C3 for aged group ≥ 42 (85.80±24.63 mg/dl) in comparison to the control (79.10±4.890 mg/dl) as demonstrated in **Table 7**, **Fig. 10** and **Fig. 11**. In the current study, the levels of C3 revealed more higher significant in the aged group ≥ 42years compared with the control of aged group while the other of aged group showed the lowest level of significant in comparison with control as seen in **Fig. 4-9**, so the high level agreed with researcher (Al-Sheikh et al.2007; Al-Samarrae,2010, Abbas et al. 2011) where they remembered that the highest levels of C3 proteins in the serum of aborted women,C3 protein considered a central particle in the system of complement that the induction represent

fundamental for all benefit functions occurred via complement system, the highest levels of C3 come from induction of pathways represented by alternative and classical (Miller et al. 2000) where C3 protein act several roles as induces the phagocytosis, provides responses the local inflammatory against pathogens and towards the responses of the adaptive immunity to take the suitable antigens for the responses of humoral immunity (AL-Fatlawi and Sultan, 2016). On the other hand, the lowest levels of C3 in the current study agreed with (Al-khayat et al. 2014), they showed that the rotation levels of C3 in the women suffering from recurrent abortion were lower than those in the pregnant women as shown in **Fig. 4-9**.

So, the tissues of the embryo are frequently develop of alloantibodies and semi-allogenic in the mother thus, the placenta is probably exposure to complement that mediated immune interface and maybe cause dangerous for loss of pregnancy (Holers et al. 2002), therefore induce components of complement which present in the normal placenta through successful pregnancy not restricted activation of complement that prevented by three proteins act as regulators and find on the membrane of trophoblast (Girardi et al. 2006). The results of this research disagreed with the other study presented by (Sugiura-Ogasawara et al. 2006, Al-khayat et al. 2014) they cleared low levels of C3 in the women serum that suffer from recurrent pregnancy loss and also they proposed that the elements of the complement play

a role in the pathogenesis of the miscarriage. The complement system participates in the form of non-specific immunity through destruction of target cell directly by the membrane attack complex, then act infiltration and induction of inflammatory cells (Xu et al. 2000), generally, the proteins of complement have a pivotal role in the protection of the embryo and maternal against agents or factors be harmful but, over- induction of complement system, causing infection by microorganisms that lead to diseases also can be a risk on the embryo (Conroy et al. 2011). Recently, complement plays an essential role in the adaptive immunity especially T cell and B cell which assist in the removing of any pathogens (Dunkelberger & Song, 2010).

## CONCLUSION

The detection of HSV-1 & HSV-2 in the aborted women's serum was higher than in pregnant women's control, and this increases the possibility of this virus as one of the major viral factors causing miscarriage. The efficiency of the rapid test (Cit EST Kit) in the identification of HSV-1 & HSV-2 was lower than that of the ELISA kit. It was evident from the low sensitivity value and increasing levels of interleukins IL-2, IL-10 and TNF-alpha was observed in aborted women than pregnant control.

## REFERENCES

- A Naqid I, H Yousif S, R Hussein N (2020). Seroprevalence of Rubella and Herpes Simplex Virus in Women with Miscarriage and Stillbirth in Zakho City, Kurdistan Region, Iraq: A Cross-Sectional Study. *Women's Health Bulletin*.7(1):8-12.
- Abbas AH, AL-Warid HS, Salloom DF (2011). Evaluation of complements serum level (C3 and C4) in pregnant women with a history of toxoplasmosis. *Journal of Biotechnology Research Center*. 5(2):12-16
- Bilal M, Khan RA, Ahmed A, Afroz S (2011) Partial evaluation of technique used in cupping. *Journal of Basic and Applied Science* 7(1): 65-68.
- Abu-Madi MA, Behnke JM, Dabritz HA (2010). Toxoplasma gondii seropositivity and co-infection with TORCH pathogens in high-risk patients from Qatar. *The American Journal of Tropical Medicine and Hygiene*.82(4):626-633.
- AL-Fatlawi SN, Sultan BA. (2016). Assessment of C3 and C4 component of the complement system in aborted women infected with Toxoplasma gondii. *Al-Qadisiyah Medical Journal*.12(22):110-114.
- Aljumaili ZKM, Alsamarai AM, Najem WS (2013). Seroprevalence of Herpes Simplex Virus Type 2 (HSV 2) in women with bad obstetric history. *American Journal of Dermatology and Venereology*.2(3):31-38.
- Al-khayat ZA, Waheda NE, Shaker NF (2014). Complement C3 and C4 Levels in Recurrent Aborting Women with or without Antiphospholipid and Anticardiolipin Autoantibodies. *Ibnosina Journal of Medicine and Biomedical Sciences*. 6(5):213-218.
- AL-Khilkhali HJ, Shubbar EE, Amjed MM, Mohsen NS, Abed Zaid ZY (2019). Using rapid and ELISA tests for detecting hepatitis C virus and human immunodeficiency virus among healthy and renal failure population. *Journal of Global Pharma Technology*.11 (02 suppl):48-55.
- Al-Samarrae EAA (2010). Study the Relationship of Toxoplasma IgG Titer with Some Other immunoglobulin and Complement Components. *Iraq Journal Community Medicine*.23(2):127-129.

- Al-Sheikh SK, Jasim AN, Ad'hiah AH (2007). Some immunologic evaluations of toxoplasmosis in Iraqi aborted females. *Baghdad Science Journal*. 4(3):444-451.
- Alzamil HA, Parade J, Fortier MA, Bernal AL (2014). Expression of the prostaglandin F synthase AKR1B1 and the prostaglandin transporter SLCO2A1 in human fetal membranes about the spontaneous term and preterm labour. *Frontiers in physiology*.5, 272.
- Arshad Z, Alturkistani A, Brindley D, Lam C, Foley K, Meinert E (2019). Tools for the diagnosis of herpes simplex virus 1/2: a systematic review of studies published between 2012 and 2018. *JMIR public health and surveillance*. 5(2),e14216.
- Azizieh FY, Raghupathy RG (2015). Tumour necrosis factor- $\alpha$  and pregnancy complications: a prospective study. *Medical Principles and Practice*.24(2):165-170.
- Cao Y, Zhang Z, Zheng Y, Yuan W, Wang J, Liang H, Shen Y (2014). The association of idiopathic recurrent early pregnancy loss with polymorphisms in folic acid metabolism-related genes. *Genes and nutrition*. 9(3), 402.
- Cheng SB, Sharma S (2015). Interleukin-10: a pleiotropic regulator in pregnancy. *American Journal of Reproductive Immunology*.73(6):487-500.
- Conroy AL, McDonald CR, Silver KL, Liles WC, Kain KC (2011). Complement activation: a critical mediator of adverse fetal outcomes in placental malaria. *Trends in parasitology*.27(7).294-299.
- Crimi S, Fiorillo L, Bianchi A, D'Amico C, Amoroso G, Gorassini F, Campagna P (2019). Herpes Virus, Oral Clinical Signs and QoL: Systematic Review of Recent Data. *Viruses*. 11(5), 463.
- Dunkelberger JR, Song WC (2010). Complement and its role in innate and adaptive immune responses. *Cell Research*.20(1):34-50.
- Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, Chrousos GP (2001). IL-12, TNF- $\alpha$ , and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *The Journal of Clinical Endocrinology and Metabolism*.86(10): 4933-4938.
- Ghaseminia, F., Daneshian, J., Soleimany, B., & Afghah, M. (2016). The Role of Stratigraphy in Growth Strata Studies: A Case Study from the Middle-Late Cretaceous Deposits in Persian Gulf, SW Iran. *International Journal of Geography and Geology*, 5(12), 249-258.
- Girardi G, Bulla R, Salmon JE, Tedesco F (2006). The complement system in the pathophysiology of pregnancy. *Molecular Immunology Journal Elsevier*.43(2):48-77.
- Grimes DA, Stuart G (2010). Abortion jabberwocky: The need for better terminology *Contraception*.81(2):93-96.
- Habib MA (2009). IFN- $\gamma$  versus IL-10 in situ expression in recurrent spontaneous abortion. *Iraqi Journal of Medical Sciences*, 7(1), 21-29.
- Holers VM, Girardi G, Mo L, Guthridge JM, Molina H, Pierangeli SS, Salmon JE (2002). Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *The Journal of experimental medicine*.195(2):211-220.
- Homes VA, Wallace JM, Gilmore WS, Mcfaul P, Alander HD (2003). Plasma levels of the immunomodulatory cytokine interleukine -10 during normal human pregnancy: a longitudinal study. *Cytokine*. 21(6):265-269.
- Hussan BM (2013). Study the prevalence of ACL, APL, CMV, HSV, Rubella, and *Toxoplasma gondii* in aborted women in Baghdad. *Medical Journal of Babylon*.10(2):455-464.
- Iche KE (2014). Seroprevalence of herpes simplex virus infections among pregnant women attending antenatal clinic in Benin, Nigeria. *International Journal Tropical Disease and Health*.4(1):70-81.
- Kamali M, Hantoushzadeh S, Boma S, Neamatzadeh H, Mazaheri M, Noor Shadkam M (2018). Association between thrombophilic genes polymorphisms and recurrent pregnancy loss
- Kapranos NC, Kotronias DC (2009). Detection of herpes simplex virus in first-trimester pregnancy losing molecular techniques. *In vivo*.23(5):839-842.
- Karad D, Kharat A (2015). Seroprevalence of Torch Infections in Bad Obstetrics History in HIV and NonHIV Women in Solapur District of Maharashtra India. *Journal Human Virol Retrovirol*.2(7):1-7
- Kaur A., and Kaur, A. (2011). Recurrent pregnancy loss: TNF- $\alpha$  and IL-10 polymorphisms. *Journal of Human Reproductive Sciences*.4(2):91-94.
- Kolawole OM, Amuda OO, Nzurumike C, Suleiman MM, Ogah JI (2016). Seroprevalence and co-infection of the human immunodeficiency virus (HIV) and herpes simplex virus (HSV) among pregnant women in Lokoja, North-Central Nigeria. *Iranian Red Crescent Medical Journal*, 18(10):e25284.
- Kolte AM, Olsen LR, Mikkelsen EM, Christiansen OB, Nielsen HS (2015). Depression and emotional stress are highly prevalent among women with recurrent pregnancy loss. *Human reproduction*.30(4): 777-782.

- Kriebs JM (2008). Understanding herpes simplex virus: transmission, diagnosis, and considerations in pregnancy management. *Journal of midwifery & women's health*.53(3): 202-208.
- Liao W, Lin JX, Leonard WJ (2013). Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity*.38(1):13-25.
- Loeffelholz MJ (2002). Rapid diagnosis of viral infections. *Laboratory Medicine*.33(4):283-286.
- Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, Newman, L. M (2015). Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. *PloS one*.10(10):e140765.
- Marchi S, Trombetta CM, Gasparini R, Temperton N, Montomoli E (2017). Epidemiology of herpes simplex virus type 1 and 2 in Italy: a seroprevalence study from 2000 to 2014. *Journal of preventive medicine and hygiene*. 58(1): 27-33.
- Mawak JD, Dashe N, Atseye AB, Agabi YA, Zakeri H (2012). Seroprevalence and co-infection of herpes simplex virus type 2 and human immunodeficiency virus in Nigeria. *Shiraz E-Medical Journal*.13(1):12-44.
- Michimata T, Sakai M, Miyazaki S, Ogasawara MS, Suzumori K, Aoki K, Saito S (2003). Decrease of T-helper 2 and T-cytotoxic 2 cells at implantation sites occurs in unexplained recurrent spontaneous abortion with normal chromosomal content. *Human Reproduction*.18(7):1523-1528.
- Miyazaki S, Tsuda H, Sakai M, Hori S, Sasaki Y, Futatani T, Saito S (2003). The predominance of Th2-promoting dendritic cells in early human pregnancy decidua. *Journal of leukocyte biology*.74(4):514-522.
- Mohaimen NA, Majid A (2011). Evaluation of IL-2, IL-8, IL-10 expression in trophoblastic tissue of women with spontaneous miscarriage infected by *Toxoplasma gondii*. *Al-Anbar Medical Journal*.9(1):50-58.
- Mohammad EAK, Salman YJ (2014). Study of TORCH infections in women with Bad Obstetric History (BOH) in Kirkuk city. *International Journal of Current Microbiology and Applied Sciences*. 3(10):700-709
- Money D, Steben M (2009). SOGC clinical practise guidelines: Guidelines for the management of herpes simplex virus in pregnancy. Number 208, June 2008. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*.104(2):167-171.
- Neamatzadeh H, Ramazani V, Kalantar SM, Ibrahimi M, Sheikhha MH (2016). Serum immune reactivity against  $\beta$ 2-Glycoprotein 1 and anti-neutrophil cytoplasmic autoantibodies by ELI- P-Complex Screening Technology in recurrent miscarriage. *Minerva Gynecol*.68(3):243-249.
- Obaid HM, Juma SA (2017). TORCH screening test in pregnant women of Kirkuk city. *Al-Mustansiriyah Journal of Science*.27(5):17-25.
- Pandey MK, Rani R, Agrawal S (2005). An update in recurrent spontaneous abortion. *Archives of gynaecology and obstetrics*. 272(2):95-108.
- Parameswaran N, Patial S (2010). Tumour necrosis factor- $\alpha$  signalling in macrophages. *Critical Reviews™ in Eukaryotic Gene Expression*. 20(2):87-103.
- Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R (2000). Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Human reproduction*.15(3):713-718.
- Rasti S, Ghasemi FS, Abdoli A, Piroozmand A, Mousavi SGA, Fakhrie-Kashan Z (2016). ToRCH "co-infections" are associated with an increased risk of abortion in pregnant women. *Congenital anomalies*.56(2):73-78.
- Rezaei A, Dabbagh A (2002). T-helper (1) cytokines increase during early pregnancy in women with a history of recurrent spontaneous abortion. *Medical Science Monitor*.8(8):607-610.
- Sadik MS, Fatima H, Jamil K, Patil C (2012). Study of TORCH profile in patients with a bad obstetric history. *Biology and Medicine*, 4(2):95-101.
- Saini V, Arora S, Yadav A, Bhattacharjee J (2011). Cytokines in recurrent pregnancy loss. *Clinica Chimica Acta*, 412(9-10), 702-708.
- Saleh DS, Kharibet KE (2015). Detection of Cytomegalovirus, Rubella virus, and IL-2 Leve Sample of Recurrently Aborted Iraqi Women. *Iraqi Journal of Science*.56(3A):1890-1894.
- Salman YJ (2007). Serological cross-reaction among causative agents of women abortions (toxoplasma, CMV, rubella, hepatitis B, and C. Tikrit Journal of Pharmaceutical Sciences. 3(20):102-111.
- Silver RM, Branch DW, Goldenberg R, Iams JD, Klebanoff MA (2011). Nomenclature for pregnancy outcomes: time for a change. *Obstetrics & Gynecology*. 118(6): 1402-1408.
- Song L, Shen L, Mandiwa C, Yang S, Liang Y, Yuan J, Wang Y (2017). Induced and spontaneous abortion and risk of uterine fibroids. *Journal of Women's Health*. 26(1):76-82.

- Sugiura-Ogasawara M, Nozawa K, Nakanishi T, Hattori Y, Ozaki Y (2006). Complement as a predictor of further miscarriage in couples with recurrent miscarriages. *Human Reproduction*. 21(10): 2711-2714.
- susceptibility in the Iran population. *A systematic review and meta-analysis Iranian Biomedical Journal*.22(2):78-89.
- Thellman NM, Triezenberg SJ (2017). Herpes Simplex Viru Establishment, Maintenance, and Reactivation: VitroModeling of Latency. *Pathogens*. 6(3), 28.
- Wald A, Ashley-Morrow R (2002). Serological testing for herpes simplex virus (HSV) –1 and (HSV)-2 infection. *Clinical infectious diseases*.35(2):173-182.
- Whitcomb BW, Schisterman EF, Klebanoff MA, Baumgarten M, Luo X, Chegini N (2008). Circulating levels of cytokines during pregnancy: thrombopoietin is elevated in miscarriage. *Fertility and sterility*. 89(6):1795-1802.
- Wood S (2011). Answering questions about herpes in pregnancy. *The Journal of perinatal education*. 20(1):61-64.
- World Health Organisation. (2012). *Safe abortion: technical and policy guidance for health systems*. 2nd Edition. Geneva.WHO.123.
- Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H (2000). A critical role for murine complement regulator carries in fetomaternal tolerance. *Science*.287(5452):498-501.
- Xu F, Sternberg MR, Kottiri BJ, McQuillan GM, Lee FK, Nahmias AJ, Markowitz LE (2006). Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *Journal of the American Medical Association*. 296(8):964-973.
- Yezlankyzy T, Niazbekov S (2015). Abortion Is the Same as a Murder of an Innocent Human Being. *International Student's Journal of Medicine*.1(4):23-30.